INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17F–104/2018/20–1–197–202
DOI: 10.17957/IJAB/15.0519
http://www.fspublishers.org

Full Length Article

Effects of Indole Acetic Acid on Growth and Endogenous Hormone Levels of Cucumber Fruit

Yongdong Sun*, Zhenxia Li, Weirong Luo, Huaicheng Chang and Guangyin Wang
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang, 453003, China
*For correspondence: sunyd2001@163.com

Abstract

Plant hormones play major role in the regulation of plant growth and development. Effects of exogenous indole acetic acid (IAA) treatment on growth and development, along endogenous hormone levels of cucumber fruit were studied using enzyme-linked immunosorbent assay (ELISA) technique. The results showed that IAA treatment had significantly increased the diameter, length and fresh weight of cucumber fruit by 14.08, 18.07 and 63.29%, respectively, compared to the unpollinated cucumber fruit, which were also positively correlated with the growing days. In addition, in comparison to the unpollinated cucumber fruit, IAA treatment remarkably improved the levels of gibberellin acid (GA_3), zeatin (ZT), abscisic acid (ABA) and GA_3/ABA ratio in the cucumber fruit. These data suggest that exogenous IAA treatment regulates the set and growth processes of cucumber fruit by controlling the endogenous hormone levels, especially by increasing GA_3/ABA. © 2018 Friends Science Publishers

Keywords: Cucumber fruit; Set and growth; IAA treatment; Endogenous hormone levels

Introduction

Cucumber (*Cucumis sativus* L.) plays an extremely important role in vegetable production and food supply (Wang *et al*., 2013). Cucumber is rich in fiber, vitamins, minerals, and amino acids, and has a high nutritional value. Cucumber also has health benefits, and is therefore very popular among the people. The annual increase in demand for cucumber in recent years has resulted in a corresponding increase in its area of cultivation. However, its production is often affected by environment stress factors such as low temperature, low light, short day, and lack of male flowers, which restrict fruit growth and development and ultimately lead to fruit abortion or a high fruit deformation rate. Some studies have shown that these environment stress factors seriously increase cell membrane permeability and malondialdehyde (MDA) content, resulting in the low yield and quality of cucumber (Mcqueen-Mason and Cosgrove, 1994).

Plant growth and development involve in a series of biochemical processes, which are regulated by plant hormones (Guney *et al*., 2016), proteins and enzymes, expression of genes (Sun *et al*., 2016) and so on. It is generally believed that fruit development is closely associated with plant hormones. Through several studies on genetic analysis of parthenocarpy (Vivian-Smith and Koltunow, 1999; Li *et al*., 2003), endogenous hormone levels during fruit growth and development (Harrison *et al*., 2001; Nin and Liu, 2002), and by artificial induction of exogenous plant hormones (Mayatal and Niimi, 1995; Catala *et al*., 2000), it was observed that auxin, gibberellin acid (GA_3), and cytokinin play a vital role in plant growth and development. These growth regulators are primarily involved in mobilizing carbohydrates, other essential nutrients, and water to the fruit. These actions in turn allow the fruit to become the metabolic center, thereby facilitating growth and development by inducing cell wall extension and cell division.

Indole acetic acid (IAA) was the first identified hormone that promotes plant growth, and is widely distributed in plants. The physiological effects of IAA can be observed at two levels. At the cellular level, it stimulates cambium cell division, whereas at the organ and whole-plant levels, its effects begin from the seedling stage to fruit maturation (Ma, 2007). Cao *et al.* (1995) studied cucumber fruit development and found that in a parthenocarpic cucumber variety, IAA levels in the fruit from 2 d to 4 d after anthesis were significantly higher than those in a non-parthenocarpic variety. Similarly, Kim *et al.* (1992) found that the auxin level in the ovary of a natural parthenocarpic cucumber variety on the day of anthesis was twice that observed in a non-parthenocarpic variety. The results of these studies indicate that cucumber fruit development is associated with a high auxin level in the ovary. However, to date, there have been very few studies on the effects of IAA treatment on cucumber fruit set and growth, and hence the regulatory mechanism of IAA remains unclear. The aim of the present study was to investigate the effects of exogenous IAA treatment on cucumber fruit growth and endogenous

To cite this paper: Sun, Y., Z. Li, W. Luo, H. Chang and G. Wang, 2018. Effects of indole acetic acid on growth and endogenous hormone levels of cucumber fruit. *Int. J. Agric. Biol*. 20: 197–202

hormone levels, which were expected to provide a theoretical basis for the further study involved in cucumber fruit set, growth and development, as well as being of practical value for the improvement of cucumber yield and quality.

Materials and Methods

Plant Material

Cucumber variety Cs0601 used as the experimental material was provided by cucumber research group in Henan Institute of Science and Technology. The enzyme-linked immunosorbent assay (ELISA) kit used for determining hormone levels was purchased from the China Agricultural University.

Plant Growth Methods and Treatments

The experiment was conducted in the lab and a plastic greenhouse of School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang, Henan Province, China. Cucumber seeds were sown in a greenhouse, and grown and managed in a conventional manner. Two days before anthesis (-2 d), male and female flowers were sealed using clip to prevent natural pollination. On the day of anthesis (0 d), female flowers were opened and 100 mg/L IAA solution was applied using a brush onto the flower corolla and ovary, which were then labelled. Followed by the IAA application the female flowers were sealed again. Ovary and fruit of unpollinated and IAA-treated cucumbers were collected at -2 d, 0 d, 1, 2, 3, 4, 5, and 6 days post anthesis (DPA). Each sample was divided into two parts. One part was stored at −80°C for subsequent determination of endogenous hormone levels, and the other part was utilized for cucumber fruit growth indexes determination.

Determination of Growth Indexes

Cucumber fruit diameter and length were measured using a vernier caliper (with a precision of 0.01 cm). Cucumber fruit fresh weight was measured using an electronic balance.

Determination of Hormone Levels

Cucumber ovary or fruit of about 0.5 g was ground in 5 mL of 80% (v/v) methanol extraction medium with 0.1% butylated hydroxytoluene and 0.06% polyvinylpyrrolidone (PVP) in an ice-cooled mortar. The homogenate was used to purify the endogenous hormones according to the method of Li (2013). The GA_3, zeatin (ZT), IAA, and abscisic acid (ABA) levels of unpollinated and IAA-treated cucumber fruit were measured using an ELISA kit (Beijing, China).

Statistical Analyses

The data of all indexes were analysed statistical using DPS 7.05 software. Analysis of variance (ANOVA) was carried out by Tukey's pair wise comparison test at 0.05 probability level, in order to determine the significant differences among treatment means.

Results

Effects of IAA Treatment on Cucumber Fruit Diameter

In our experiment, effects of the growing days were remarkable on fruit diameter. In both unpollinated and IAA-treated cucumber fruit, fruit diameter showed an upward trend with the growing days, and was positively correlated with the number of growing days. At -2 d, the fruit diameter was 0.66 cm. At 6 DPA, the fruit diameter of unpollinated cucumber had reached 1.37 cm, whereas that of IAA-treated cucumber had reached 1.93 cm. From 2 to 6 DPA, IAA treatment significantly increased fruit diameter, compared to the unpollinated. Overall, fruit diameter of IAA-treated cucumber was all larger than that of unpollinated cucumber, with an average increase of 14.08%, suggesting that exogenous IAA treatment promoted cucumber fruit cell division and increased fruit diameter (Fig. 1).

Effects of IAA Treatment on Cucumber Fruit Length

With an increase in the growing days, there was an upward trend in fruit length of both unpollinated and IAA-treated cucumbers, and this increase was positively correlated with the number of growing days. At -2 d, fruit length was 1.8 cm, which was shortest. A more rapid increase was observed at 6 DPA. At 6 DPA, fruit length had reached 3.80 cm and 5.23 cm for unpollinated and IAA-treated cucumbers, respectively. From 1 to 6 DPA, IAA treatment remarkably increased the fruit length, compared to the unpollinated. Fruit length of IAA-treated cucumber was all obviously larger than that of unpollinated cucumber, with an average increase of 18.07%. Therefore, exogenous IAA treatment can increase cucumber fruit length and promote fruit growth (Fig. 2).

Effects of IAA Treatment on Fresh Weigh of Cucumber Fruit

As shown in Fig. 3, fruit fresh weight of unpollinated and IAA-treated cucumbers showed an overall upward trend. From -2 d to 0 d, fresh weight of cucumber fruit did not change markedly. From 1 to 6 DPA, effects of the growing days were remarkable on fresh weight of cucumber fruit. There was a significant difference between unpollinated and IAA-treated cucumbers. From 3 to 6 DPA, IAA treatment significantly increased fresh weight of cucumber fruit, compared to the unpollinated. At 6 DPA, the fresh weight of unpollinated cucumber fruit had increased to 3.36 g, whereas that of IAA-treated cucumber had increased to 7.68 g, which was 2.29 times greater than that of unpollinated cucumber.

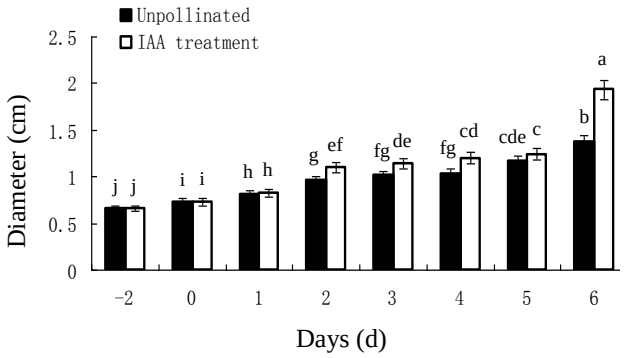

Fig. 1: Changes of diameter of cucumber fruit during the growth

Each bar indicates mean of three replications. Bars sharing the same letter, for a index, don't differ significantly at $P \leq 0.05$

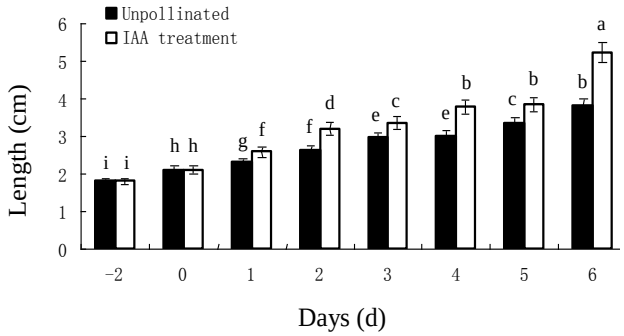

Fig. 2: Changes of length of cucumber fruit during the growth

Each bar indicates mean of three replications. Bars sharing the same letter, for a index, don't differ significantly at $P \leq 0.05$

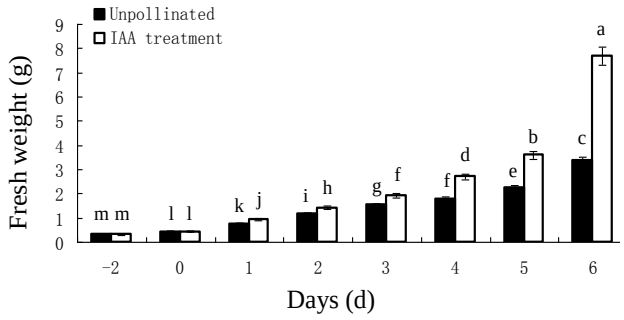

Fig. 3: Changes of fruit fresh weight of cucumber fruit during the growth

Each bar indicates mean of three replications. Bars sharing the same letter, for a index, don't differ significantly at $P \leq 0.05$

Furthermore, fresh weight of IAA-treated cucumber fruit was all obviously larger than that of unpollinated cucumber fruit, with an average increase of 63.29%.

Effects of IAA Treatment on GA_3 Level in Cucumber Fruit

As presented in Fig. 4, with an increase in the growing days,

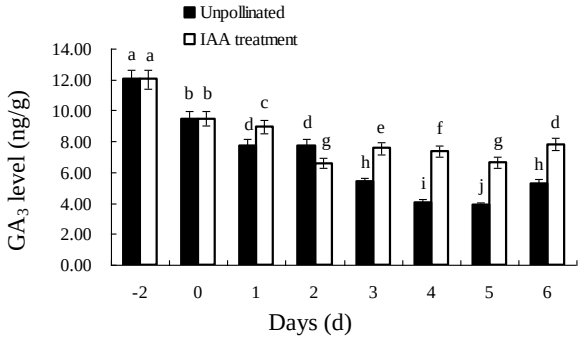

Fig. 4: Changes of GA_3 level in cucumber fruit during the growth

Each bar indicates mean of three replications. Bars sharing the same letter, for a index, don't differ significantly at $P \leq 0.05$

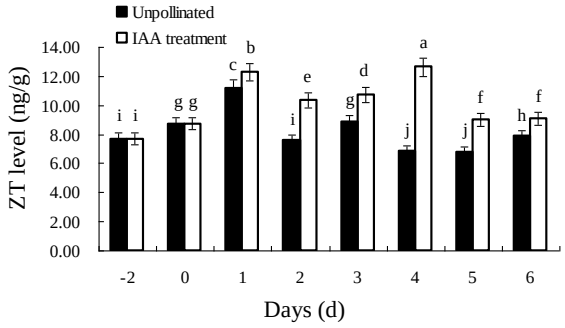

Fig. 5: Changes of ZT level in cucumber fruit during the growth

Each bar indicates mean of three replications. Bars sharing the same letter, for a index, don't differ significantly at $P \leq 0.05$

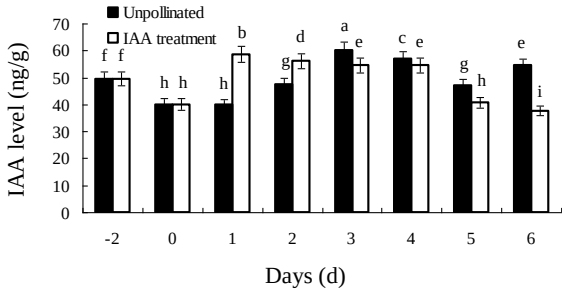

Fig. 6: Changes of IAA level in cucumber fruit during the growth

Each bar indicates mean of three replications. Bars sharing the same letter, for a index, don't differ significantly at $P \leq 0.05$

there was an overall downward trend in the fruit GA_3 levels of both unpollinated and IAA-treated cucumbers, the level being highest (12.04 ng/g) at -2 d. From 1 to 6 DPA, there was a significant difference in terms of GA_3 level between unpollinated and IAA-treated cucumbers. GA_3 level of unpollinated cucumber fruit decreased more rapidly than that of IAA-treated cucumber fruit, with decreases of 42.28% and 31.06% being observed at 6 DPA, respectively. Thus, IAA treatment can significantly slow down the

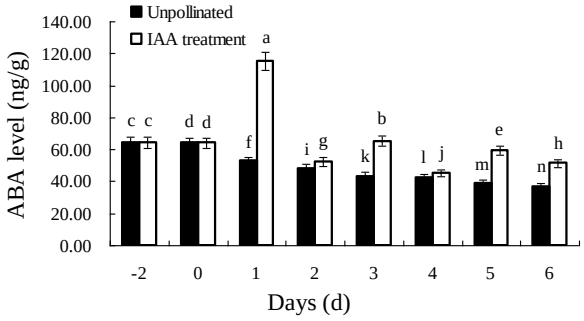

Fig. 7: Changes of ABA level in cucumber fruit during the growth

Each bar indicates mean of three replications. Bars sharing the same letter, for a index, don't differ significantly at $P \leq 0.05$

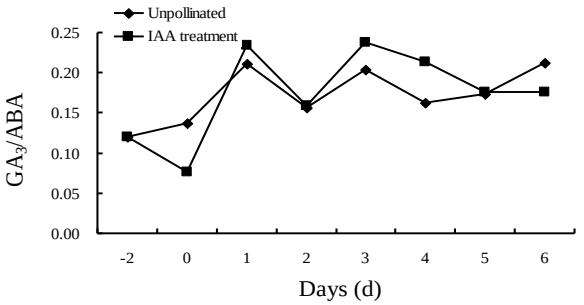

Fig. 8: Changes of GA_3/ABA in cucumber fruit during the growth

decrease in GA_3 level of cucumber fruit.

Effects of IAA Treatment on ZT Level in Cucumber Fruit

From -2 d to 1 DPA, ZT levels of unpollinated cucumbers increased from 7.70 to 11.18 ng/g, an increase of 45.19%, but thereafter gradually declined to 7.87 ng/g at 6 DPA. From -2 d to 4 DPA, ZT levels of IAA-treated cucumber fruit increased and reached a maximum level of 12.65 ng/g at 4 DPA, an increase of 64.29%. Thereafter, ZT level began to decline, reaching 9.06 ng/g at 6 DPA. From 1 to 6 DPA, as for ZT level, there was a significant difference between unpollinated and IAA-treated cucumbers. ZT levels of IAA-treated cucumber fruit were overall significantly higher than those of unpollinated cucumber fruit (Fig. 5). This result suggested that exogenous IAA treatment could significantly increase the endogenous ZT level in cucumber fruit.

Effects of IAA Treatment on IAA Level in Cucumber Fruit

As given in Fig. 6, from 1 to 6 DPA, there was a significant difference in term of IAA level between unpollinated and IAA-treated cucumbers. From 1 to 2 DPA, IAA levels of IAA-treated cucumber fruit were significantly higher than those of unpollinated cucumber fruit. However, from 3 to 6 DPA, the endogenous IAA levels of IAA-treated cucumber

Table 1: Average levels of hormones during -2 d to 6 DPA

Treatment	GA_3/ABA	ZT/ABA	IAA/ABA	ABA/(GA_3+ZT+IAA)
Unpollinated cucumber fruit	0.171:1	0.139:1	1.068:1	0.757:1
IAA treatment cucumber fruit	0.175:1	0.136:1	0.808:1	0.959:1

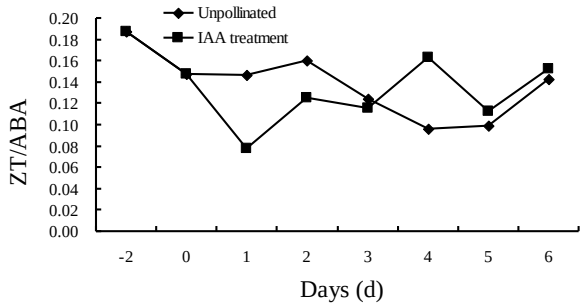

Fig. 9: Changes of ZT/ABA in cucumber fruit during the growth

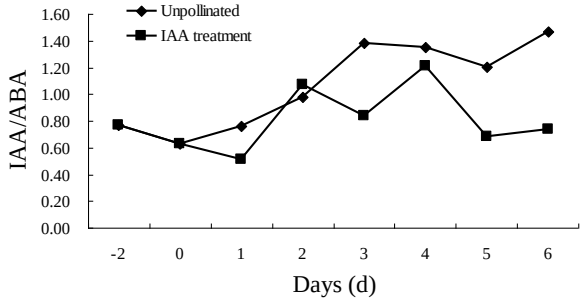

Fig. 10: Changes of IAA/ABA in cucumber fruit during the growth

fruit decreased slowly and were lower than those of unpollinated cucumber fruit.

Effects of IAA Treatment on ABA Level in Cucumber Fruit

From 1 to 6 DPA, there was a significant difference in term of ABA level between unpollinated and IAA-treated cucumbers. And ABA levels of IAA-treated cucumbers were all higher than those of unpollinated cucumbers. ABA level of unpollinated cucumber fruit at -2 d was 64.36 ng/g and decreased to 37.04 ng/g at 6 DPA, a decrease of 42.45%. ABA level of IAA-treated cucumber fruit, reached a maximum of 115.19 ng/g at 1 and thereafter gradually decreased. At 6 DPA, ABA level had reached 51.33 ng/g, which was still higher than that of unpollinated cucumber fruit (Fig. 7).

Effects of IAA on the Ratios of GA_3/ABA, ZT/ABA and IAA/ABA in Cucumber Fruit

From 1 to 5 DPA, the GA_3/ABA ratio of IAA-treated

cucumber fruit was higher than that of unpollinated cucumber fruit (Fig. 8). IAA treatment decreased the ZT/ABA ratio at 1 DPA to 3 DPA, and increased at 4 to 6 DPA (Fig. 9). In addition, IAA treatment decreased the IAA/ABA ratio throughout the process of fruit growth and development, except 2 DPA (Fig. 10), suggesting that the balance between hormones plays an important regulatory role in fruit growth and development. As shown in Table 1, the ABA/(GA_3+ZT+IAA) ratio was less than 1 in both unpollinated and IAA-treated cucumber fruit, suggesting that the hormones of cucumber fruit during this period were mainly growth-promoting hormones.

Discussion

It is currently believed that IAA mainly functions by promoting cell elongation and volume increase, and plays an important role in the growth of plant tissues. The results of this experiment showed that from -2 d to 6 DPA, the fruit diameter, length, and fresh weight in both unpollinated and IAA-treated cucumber fruit displayed upward trends, and were positively correlated with the growing days. In addition, fruit diameter, length, and fresh weight in IAA-treated cucumber fruit were all significantly higher than those in unpollinated cucumber fruit, with average increases of 14.08, 18.07 and 63.29%, respectively. Thus, exogenous IAA treatment can increase cucumber fruit diameter, length, and fresh weight, and induce fruit expansion, and play an important regulatory role in cucumber fruit growth (Yang *et al*., 2014).

Plant hormones regulate fruit growth and development (Crane, 1964; Srivastava and Handa, 2005; Sun *et al*., 2017). GA_3 is a growth-promoting hormone that accelerates cell differentiation, longitudinal elongation of mature cells, and intermodal cell elongation. IAA and GA_3 have synergistic effects. ZT mainly functions in promoting callus germination and fruit set. ABA is a growth inhibitory hormone and promotes dormancy. There is thus antagonism between ABA and GA_3 (Skriver *et al*., 1991). Our study found that GA_3, ZT and ABA levels were remarkably higher in IAA-treated cucumber fruit than those in unpollinated cucumber fruit, which suggests that exogenous IAA treatment can affect endogenous hormone levels, thereby promoting cucumber fruit set and growth. Fruit growth and development are associated with not only one type of hormones, but are related to the coordination and balance among hormones (Xu *et al*., 2015). Unbalanced levels of ABA and other hormones have been shown to result in ovary shedding (Chen *et al*., 2000). Zhang *et al.* (2009) found that when the ABA/(GA_3+ZT+IAA) ratio was less than 1, fruit contained mainly endogenous growth-promoting hormones, allowing successful fruit set of the ovary. Our study showed that the ABA/(GA_3+ZT+IAA) ratio was less than 1, which is consistent with the findings of Zhang *et al.* (2009). This suggested that IAA treatment could improve cucumber fruit set rate and promote fruit growth. This study also found that IAA treatment increased the GA_3/ABA ratio, indicating that during the period of fruit set and growth, the rate of GA_3 increase exceeded that of ABA, which suggests that IAA treatment promote fruit set and growth by increasing GA_3/ABA.

Conclusion

In this paper, effects of exogenous IAA treatment on growth, and endogenous hormone levels of cucumber fruit during the fruit set and growth were investigated, and the results showed that IAA treatment significantly increased the diameter, length and fruit fresh weight of cucumber fruit, and improved GA_3/ABA ratio in fruit. It suggests that exogenous IAA treatment regulates the set and growth processes of cucumber fruit by controlling the endogenous hormone levels, especially increasing GA_3/ABA.

Acknowledgements

Research sponsored by the National Natural Science Foundation of China (31401860 and U1204322) and Program for Science & Technology Innovation Talents in Universities of Henan Province (17HASTIT040).

References

Cao, P.S., M.M. Miao, X.H. Chen and Q. Xu, 1995. Changes of endogenous hormone contents and peroxidase activity of ovary and their relation to parthenocarpy in cucumber (*Cucumis sativus* L.). *Plant Physiol. Commun*., 34: 347–349

Catala, C., J.K. Rose and A.B. Bennett, 2000. Auxin-regulated genes encoding cell wall-modifying proteins are expressed during early tomato fruit growth. *Plant Physiol*., 122: 527–534

Chen, F.H., W.Y. Zhang and X. Yu, 2000. Effect of growth regulators spray on postharvest berry drop of the grape cultivar 'Wuhebai'. *J. Xinjiang Agric. Univ*., 23: 1–5

Crane, J., 1964. Growth substances in fruit setting and development. *Annu. Rev. Plant Physiol*., 15: 303–326

Guney, K., M. Cetin, H. Sevik and K.B. Guney, 2016. Guney, influence of germination percentage and morphological properties of some hormones practice on *Lilium martagon* L. seeds. *Oxidation Commun*., 39: 466–474

Harrison, E.P., S.J. Mcqueen-Mason and K. Manning, 2001. Expression of six expansin genes in relation to extension activity in developing strawberry fruit. *J. Exp. Bot*., 52: 1437–1446

Kim, I.S., H. Okubo and K. Fujieda, 1992. Endogenous levels of IAA in relation to parthenoearpy in cucumber (*Cucumis sativus* L.). *Sci. Hortic*., 52: 1–8

Li, Y.W., 2013. *The Dynamic Changes in Endogenesis Hormone during Eggplant Parthenocarpic Fruit Development and Analysis of Differentially Expressed Genes.* China Academy of Agriculture Science, Beijing, China

Li, Y., L. Jones and S. Mcqueen-Mason, 2003. Expansins and cell growth. *Curr. Opin. Plant Biol*., 6: 603–610

Ma, H.Y., 2007. *Changes of Endogenous Hormones in Grapevine during its Development.* Northwest Agriculture Forest University, Yangling, China

Mayatal, Y. and Y. Niimi, 1995. Synthetic cokinin-1-(2-chloro-4-pyridyl)-phenyluera (CPPU) promotes fruit set and induces parthenocarpy in watermelon. *Amer. Soc. Hortic. Sci*., 120: 997–1000

Mcqueen-Mason, S. and D.J. Cosgrove, 1994. Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. *Proc. Natl. Acad. Sci. USA*, 91: 6574–6578

Nin, L. and H.X. Liu, 2002. Effect of pollination on the change of endohormones in the fruit of shatianyou pomelo variety. *J. Fruit Sci*., 19: 27–31

Skriver, K., F.L. Olsen, J.C. Rogers and J. Mundy, 1991. Cis-acting DNA elements responsive to gibberellin and its antagonist abscisic acid. *Proc. Natl. Acad. Sci. USA*, 88: 7266–7270

Srivastava, A. and A.K. Handa, 2005. Hormonal regulation of tomato fruit development: a molecular perspective. *J. Plant Growth Regul*., 24: 67–82

Sun, Y.D., L. Ni, S.Y. Sun and W.R. Luo, 2016. Effects of expression of Csexpansin 10 (*CsEXP* 10) gene on the growth and antioxidation enzymes activities of tomato. *Oxidation Commun*., 39: 1293–1299

Sun, Y.D., W.R. Luo, Z.X. Li and X.Z. Li, 2017. Endogenous hormones levels and *Csexpansin* 10 gene expression in the fruit set and early development of cucumber. *J. Chem. Soc. Pak*., 39: 59–64

Vivian-Smith, A. and A.M. Koltunow, 1999. Genetic analysis of growth-regulator-induced parthenocarpy in *Arabidopsis*. *Plant Physiol*., 121: 437–451

Wang, J., S.J. Zhang, X. Wang, L.N. Wang, H.N. Xu, X.F. Wang, Q.H. Shi, M. Wei and F.J. Yang, 2013. *Agrobacterium*-mediated transformation of cucumber (*Cucumis sativus* L.) using a sense mitogen-activated protein kinase gene (*CsNMAPK*). *Plant Cell Tiss. Org. Cult*., 113: 269–277

Xu, Z.M., E.H. Zhang, Y.A. Cheng and Q.S. Ma, 2015. Relationships between endogenous hormone contents and cytoplasmic male sterility in cabbage. *North. Hortic*., 8: 1–5

Yang, X.W., G.Q. Zheng, J. Yang, X. Xu, D. Lu and L. Yang, 2014. Changes in endogenous hormone contents and cell wall component, degrading enzyme activity and their relation in Lycium barbarum, *Bull. Bot*., 49: 30–40

Zhang, W.C., Y.T. Wei, J. Wang, M. He, P. Tang and X.J. Du, 2009. The relationship between endogenous hormone and oarthenocarpy in the eggplant fruitlets. *J. Shenyang Agric. Univ*., 40: 3–6

(Received 22 August 2017; Accepted 04 October 2017)